CHAPTER 2

LITERATURE REVIEW

2.1 Botanical aspect of *Senna alata* (L.) Roxb.

Senna alata (L.) Roxb. (Fig.2.1) (previously name *Cassia alata* L.) belongs to Leguminosae family and widely distribute in parts of Thailand. It has many English names including Candle bush, Candlestick senna, Christmas candle, Ringworm bush, Ringworm senna and Seven golden candlesticks. Its local Thai names are, Chum-het-yai (Central), Khi-khak (Northern), Lap-muen-luang (Northern) and Mak-kaling-thet (Northern).

Senna alata grows well in full sun in a wide range of soils, which retain moisture adequately. This plant grows particularly aggressively in areas where there is a high water table and prefers not too dry habitats. The plant is a shrub normally 1 - 2 m high but sometimes up to 5 m high and has horizontally spread branches. Stipules are auriculate-deltoid, 6 - 15 mm and persistent. Leaves spirally or alternate arranged; paripinnate with 8 - 20 pairs of leaflets; petiole robust, 2 - 3 cm; rachis 30 - 60 cm. Leaflets oblong-obovate or oblong-elliptic, 5 - 15 cm long and 3 - 7 cm wide; base rounded or cordate; apex rounded or almost notched, with a short sharp point; margin entire; petiolules robust, 2 - 3 mm. Inflorescence racemose, axillary and terminal, dense robust, many-flowered, 20 - 50 cm long and 3 - 4 cm wide; bracts strobilate, at first enveloping the flowers, yellow, broadly ovate, caducous, 2 - 3 cm long and 1 - 2 cm wide; flower bright yellow; pedicels 5 - 10 mm. Sepals 5, orange-yellow, oblong, subequal, 1 - 2 cm long and 6 - 7 mm wide. Petals 5, subequal, bright yellow, ovate-orbicular, 1.6 - 2.4 cm long and 1 - 1.5 cm wide, short-clawed. Stamens 9 - 10; 2 large with stout filaments 4 mm long and anthers 12 - 13 mm opening by apical pores; 4 with filaments 2 mm long and anthers 4 - 5 mm opening with apical pores; reduced stamens 3 - 4. Superior ovary puberulous, ovules many;

style filiform; stigma small. Pod Dehiscent, sharply tetragonal, blackish, winged, 10 – 15 cm long and 1.5 – 2 cm wide, wings 4 – 8 mm wide. Seeds many up to 50, shining, flattened, slightly quadrangular, 7 – 8 long and 5 – 8 mm wide (Subcommittee on the Establishment of the Thai Harbal Pharmacopoeia, 1998).

Fig 2.1 *Senna alata* (L.) Roxb.

2.2 Chemical constituents of *Senna alata*

The groups of secondary metabolites commonly found in *S. alata* are anthraquinones, flavonoids and fatty acids. Lists of compounds that have been found in various parts of *S. alata* are shown in Table 2.1. The structure of the anthraquinone derivatives of interest to the current study are given in Fig. 2.2.

Table 2.1 Chemical constituents of various parts of *Senna alata*

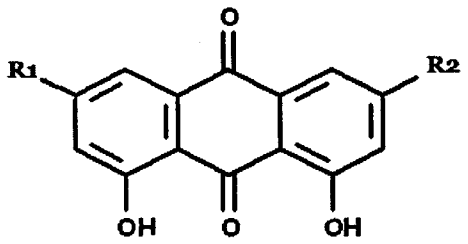
Plant part	Category	Chemical substance	Reference
Leave	Flavonoid glycoside	kaempferol-3-O-gentiobioside	Moriyama *et al.*, 2003
	Flavonoid	kaempferol	Rao *et al.*, 1975
	Anthraquinone	aloe-emodin	Rao *et al.*, 1975
		rhein	Hauptmann and Nazario, 1950
		isochrysophanol	Smith and Ali, 1979
		physcion-L-glucoside	Smith and Ali, 1979
		chrysophanol	Villaroya and Bernal-Santos, 1976
		sennosides A, B, C, and D	Harrison and Garro, 1977
		emodin	Villaroya and Bernal-Santos, 1976
	Anthraquinone glycoside	rhein-8-glucoside	Rai, 1978
		aloe-emodin-8-glucoside	Rai, 1978
	Sterol	sitosterol	Rao *et al.*, 1975
	Polyphenol	2,3,7-tri-O-methylellagic acid	Alam *et al.*, 2003

Table 2.1 (cont.)

Plant part	Category	Chemical substance	Reference
Flower	Flavonoid glycoside	kaempferol-3-O-gentiobioside	Moriyama *et al.*, 2003a
Seed	Polyalcohols	glycerol	Singh, 1998
		erythritol	Singh, 1998
	Carbohydrate	galactomannans	Gupta *et al.*, 1987
	Flavonoid glycoside	chrysoeriol-7-O-(2"-O-β -D-mannopyranosyl)-β-D-allopyranoside	Dipti, 1991
		rhamnetin-3-O-(2"-O-β-D-mannopyranosyl)-β-D-allopyranoside	Dipti, 1991
	Sterol	β-sitosterol	Miralles and Gaydou, 1986
		sitostrol	Singh and Tiwari, 1943
		stigmasterol	Miralles and Gaydou, 1986
		campesterol	Miralles and Gaydou, 1986
		22-dihydrospinasterol	Miralles and Gaydou, 1986
		28-isoavenasterol	Miralles and Gaydou, 1986
	Fatty acid	linoleic acid	Singh and Tiwari, 1943
		oleic acid	Singh and Tiwari, 1943; Morah and Otumu, 1991
		palmitic acid	Singh and Tiwari, 1943; Morah and Otumu, 1991
		lignoceric acid	Singh and Tiwari, 1943
		isopalmitic acid	Morah and Otumu, 1991

Table 2.1 (cont.)

Plant part	Category	Chemical substance	Reference
		palmitoleic acid	Morah and Otumu, 1991
		myristoleic acid	Morah and Otumu, 1991
		tridecanoic acid	Morah and Otumu, 1991
		myristic acid	Morah and Otumu, 1991
	Anthraquinone	chrysophanol	Morah and Otumu, 1991
		emodin	Morah and Otumu, 1991
		aloe-emodin	Morah and Otumu, 1991
		rhein	Morah and Otumu, 1991
Root	Anthraquinone	alquinone	Yadav and Kalidhar, 1994
Stem	Flavonoid glycoside	kaempferol-3-O-gentiobioside	Moriyama *et al.*, 2003a
	Anthraquinone glycoside	5-hydroxy-2-methylanthraquinone-1-O-rutinoside	Rai and Prasad, 1994
	Anthraquinone	emodin	Kelly *et al.*, 1994
		1,5-dihydroxy-2-methlanthraquinone	Rai and Prasad, 1994
	Anthrone	3-formyl-1,6,8,10-tetrahydroxyanthrone (alarone)	Hemlata and Kalidhar, 1994.
	Sterol	β-sitosterol	Rai and Prasad, 1994
Fruit	Anthraquinone	rhein	Rai, 1978
		aloe-emodin	Rai, 1978
		emodin	Rai, 1978



Aloe-emodin	: R1 = CH_2OH; R2 = H
Chrysophanol	: R1 = CH_3; R2 = H
Emodin	: R1 = CH_3; R2 = OH
Physcione	: R1 = CH_3; R2 = OCH_3
Rhein	: R1 = COOH; R2 = H

Fig 2.2 Chemical structures of anthraquinones

2.3 Biosynthesis of anthraquinone

Anthraquinones have long been established. Chrysophanol or chrysophanic acid from rhubarb and cascara; aloe-emodin from rhubarb and senna; rhein from rhubarb and senna; emodin from rhubarb and cascara were recognized as forming a natural group of purgative drugs. In dicotyledons they occur in the Rubiaceae, Leguminosae, Polygonaceae, Rhamnaceae, Ericaceae, Euphorbiaceae, Lythraceae, Saxifragaceae, Scrophulariaceae and Verbenaceae (Evans, 2002).

Anthracenes, mostly at the quinone oxidation level are commonly found in microorganisms, plants, and lower animals. Such compounds are mainly of polyketide origin and are derived from the cyclisation of and octaketide chain in all the cases so far examined. The tricyclic skeleton can lose the 3-carboxylic group, producing anthracene derivatives with 15 carbon atoms. Such derivatives are common and are found in a wide range of fungi (Manitto and Sammes, 1981).

Endocrocin is an anthraquinone found in species of *Penicillum* and *Aspergillus* fungi. Folding a polyketide containing eight C_2-units forms the periphery of the carbon skeleton (Fig 2.3). Three aldol-type condensations would give a hypothetical

intermediate **1**. Except for crucial carbonyl oxygen in the center, endocrocin results by enolization reaction, one of which involves the vinylogous enolization. The additional carbonyl oxygen must be introduced at some stage during the biosynthesis by an oxidative process. Emodin, a metabolite of some *Penicillium* species, but also found in higher plants, e.g. *Rhamnus* and *Rumex* species, would appear to be formed from endocrocin by a simple decarboxylation reaction. This is facilitated by the adjacent phenol function. *O*-methylation of emodin would then lead to physcione (Dewick, 2001).

Islandicin is another anthraquinone pigment produced by *Penicillium islandicum*, and differs from emodin in two ways. One hydroxyl is missing, and a new hydroxyl has been incorporated adjacent to the methyl. Without any avidence for the sequence of such reactions, the structure of intermediate **2** shows the result of three aldol condensations and reduction of a carbonyl. A dehydration reaction, two oxidations, and a decarboxylation are necessary to attain the islandicin structure (Dewick, 2001).

In chrysophanol, aloe-emodin and rhein, the same oxygen function is lost by reduction as in islandicin, and decarboxylation also occurs. The three compounds are interrelated by a sequential oxidation of the methyl in chrysophanol to a hydroxymethyl in aloe-emodin and a carboxyl in rhein (Dewick, 2001).

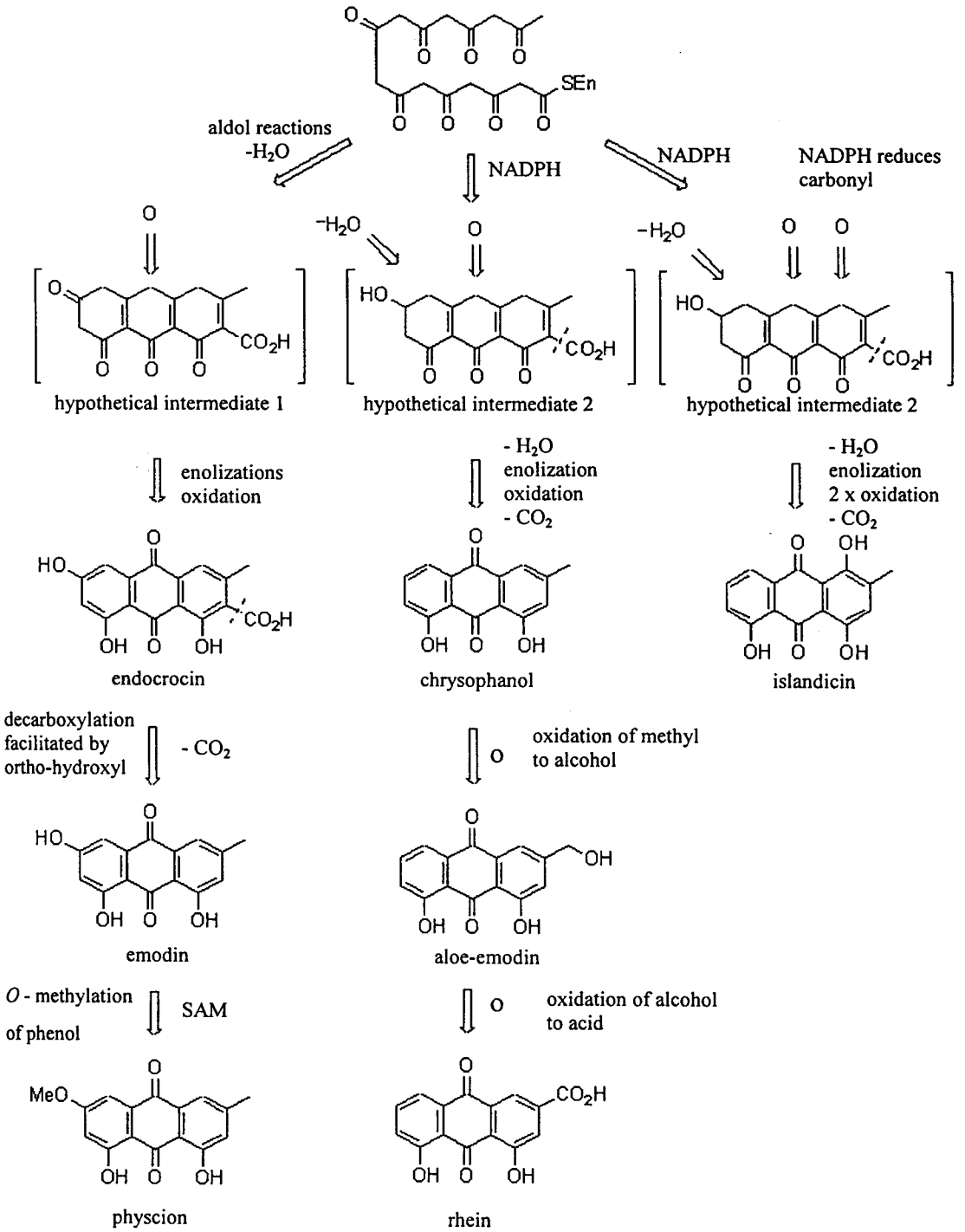


Fig 2.3 Biogenesis pathway of anthraquinones (Dewick, 2001)

2.4 Biological activity

2.4.1 *Senna alata* crude extracts

Senna alata has long been used as a folk medicine in Thailand. The two properties mostly acknowledged for a long time are laxative and topical antifungal for the treatment of dermatophyte infection (สำนักงานคณะกรรมการการสาธารณสุขมูลฐาน, 2541; Farnsworth and Bunyapraphatsara, 1992). Both properties of *S. alata* have been scientifically proven *in vitro* and in animal studies. It has been demonstrated that *S. alata* leaf extract in a dose of 800 mg/kg possessed a laxative activity in rat. (Elujoba *et al.*, 1989; Ogunti and Elujoba, 1993). A Clinical study has indicated that the therapeutic efficacy of *S. alata* is higher than placebo and mist alba (Thamlikitkul *et al.*, 1990). Hence, *S. alata* has been approved as a laxative drug in the Thai National List of Essential Drug 1999 (National Drug Committee, 1999). However, signs of toxicity including loss of appetite, emaciation and loss of weight have been reported in treated rats. The toxicity has been reported to be due to the saponin content in the plant extracts (Sodipo *et al.*, 1998).

Many studies have shown the antifungal activity of the extracts from various parts *S. alata* against dermatophytes (Fuzellier *et al.*, 1982; Palanichamy and Nagarajan, 1990b; Villasenor *et al.*, 2002). The leaf extract showed antifungal activity against *Trichophyton mentagorphytes*, *T. rubrum* and *Microsporum gypseum* with minimum inhibitory concentration (MIC) values of 125 mg/ml, and against *Microsporum canis* with MIC value of 62.5 mg/ml (Ibrahim and Osman, 1995). The inhibition can be observed on the macroconidia of *Microsporum gypseum*, in which the drug caused resulted in structural degeneration beyond repair. The mechanism of inhibition can be related to the cell leakage as observed by irregular, wrinkle shape and loss in rigidity of the macroconidia (Ibrahim and Osman, 1995). It has been reported that the antifungal activity of *S. alata* extract may be due to the presence of chrysophanol (Palanichamy and Nagarajan, 1990b).

However, *S. alata* extract exhibited a weak activity or negligible activity against *Candida albicans* (Mastura and Khozirah, 2000). The MIC and minimum fungicidal concentration (MFC) of the water leaf extract of *S. alata* against *C. albicans* were 0.39 mg/ml and 60 mg/ml, respectively (Crockett *et al.*, 1992). In addition, the ethanol and water extracts from the barks of *S. alata* exhibited antifungal activity against *C. albicans*, but the yeast was not affect by the extracts of the leaves (Somchit *et al.*, 2003). A clinical study on the treatment of *Pityriasis versicolor* using the aqueous extract of *S. alata* leaves has been reported. A total of 200 male and female patients (between 16 and 60 years old) were treated with a concentrated solution (100% aqueuous extract). The results indicated that a single application of the extract was sufficient to cure the fungal disease and to protect the patients from recurrence for up to one year (Damodaran and Venkataraman, 1994).

Several studies also indicated that *S. alata* extracts possessed antibacterial activity (Somchit *et al.*, 2003; Adedayo *et al.*, 2001; Khan *et al.*, 2001; Mastura and Khozirah, 2000; Sakharkar and Pati, 1998). The aqueous and alcohol extracts from the leaves of *S. alata* were effective in reducing the weight of *E. coli*-inoculated rat intestinal segments (Medina *et al.*, 2001). The MIC, minimum bactericidal concentration (MBC) and IC_{50} for the water extracts of *S. alata* against *E. coli* were 1.6 mg/ml, 60 mg/ml and 31 mg/ml, respectively (Crockett *et al.*, 1992).

In addition, there have been many reports on other pharmacological activities of *S. alata* extracts, including antitumor (Balboa *et al.*, 1992; Serrame *et al.*, 1995), choleretic (Assane *et al.*, 1993), hepato-protective (Effraim *et al.*, 1999), hypoglycemic (Palanichamy *et al.*, 1988; Villasenor *et al.*, 2002), antimutagenic (Villasenor *et al.*, 2002), antiinflamatory (Palanichamy and Nagarajan, 1990a; Villasenor *et al.*, 2002; Moriyama *et al.*, 2003b), antioxidant (Panichayupakaranant and Kaewsuwan, 2004). Recently, the extracts of the *S. alata* have been used in cosmetic and/or dermatological skin care products (Palanichamy *et al.*, 1991; Danoux *et al.*, 2002).

S. alata has also been used in animals. For example, ointments containing ethanol extracts from the leaves, are used as topical treatments for chronic crusty or acute lesions of dermatophilosis in bovine animals. The healed animals became free of dermatophilosis without recurrence for more than 3 years (Ali-Emmanuel *et al.*, 2003).

2.4.2 Aloe-emodin

It has been reported that aloe-emodin exhibits antibacterial activity against *Staphylococcus aureus* and *Streptococcus viridans*, with MIC values of 6.25 - 12.5 and 25 - 50 μg/mL, respectively. In addition aloe-emodin showed antibacterial activity against *Helicobacter pylor*i through the inhibition of arylamine N-acetyltransferase (Cai and Chen, 1988; Wang *et al.*, 1990; Liu *et al.*, 1996; Wang *et al.*, 1998).

Aloe-emodin showed anticancer activity against P388 leukemia in mice and human oral squamous cell carcinoma (HSC-2) and salivary gland tumor cell lines (HSG) (Kupchan and Karim, 1976; Shi *et al.*, 2001). There are many mechanisms involved in anticancer activity of aloe-emodin, including inhibition the non-covalent binding of bisbenzimide Hoechst 33342 to isolated DNA and in mouse lymphoma L5178Y cells comparable to the topoisomerase II inhibitor (Mueller and Stopper, 1999); induction of apoptosis in human lung squamous carcinoma cell (CH27) and human lung non-small cell carcinoma cell (H460) by nuclear morphological changes and DNA fragmentation (Lee, 2001) and expression of p38 protein in human lung nonsmall carcinoma cells (H460) (Kuo *et al.*, 2002; Yeh *et al.*, 2003); inhibition of N-acetylation and DNA adduct of 2-aminofluorene and arylamine N-acetyltransferase gene expression in mouse leukemia L 1210 cells (Chung *et al.*, 2003); reduction growth inhibitory and pro-apoptotic activity in p53 mutant cells (Pecere *et al.*, 2003). Aloe-emodin has a specific *in vitro* and *in vivo* antineuroectodermal tumor activity (Pecere *et al.*, 2000). In contrast, it has been reported that *in vivo* treatment of primary rat hepatocytes with aloe-emodin resulted in a 2-3 fold increase of DNA

synthesis (Wolfle *et al.*, 1990). *In vitro* induction of cell number increasing in human colorectal cancer cell line (SW480) by aloe-modin have also been observed (Schorkhuber *et al.*, 1998).

Moreover, aloe-emodin has been found to have purgative activity (Fairbairn and Moss, 1970; D'angelo, 1993); antiviral activity to enveloped viruses against herpes simplex virus type 1 and type 2, varicella-zoster virus, pseudorabies virus and influenza virus by disrupted enveloped of virus (Sydiskis *et al.*, 1991); antioxidation activity (Malterud *et al.*, 1993); anti-inflammatory (Yamamoto *et al.*, 1991; Yin and Xu, 1998; Arosio *et al.*, 2000); antiprotozoal activity (Camacho *et al.*, 2000) and antihepatotoxic (Woo *et al.*, 2002).

However, mutagenic activity of aloe-emodin has been reported. Genotoxicity of aloe-emodin has been reported in the Salmonella reverse mutation test and hypoxanthine-guanine-phosphoribosyl transferase locus in Chinese hamster fibroblasts (V79) (V79-HGPRT) mutagenicity assay. Aloe-emodin induced mutations in mouse lymphoma cells (L5178Y) by inhibition of the topoisomerase II-mediated decatenation (Mueller *et al.*, 1996). In contrast, some studies e.g. *in vitro* gene mutation test with V79 cells (HGPRT test); DNA synthesis in hepatocytes of male Wistar rats demonstrated that aloe-emodin did not act as a mutagen (Heidemann *et al.*, 1993; Westendorf *et al.*, 1990).

2.4.3 Chrysophanol

Chrysophanol has been reported to possess anticancer activity against various tumor cell lines, including mouse lymphoma L5178Y cells (Mueller *et al.*, 1998), mouse P388 leukemia, V-79 cells (Abd El-Fattah *et al.*, 1997) and human hepatoma cell line (Hep G2)(Wu *et al.*, 2001). However, *in vivo* treatment of primary rat hepatocytes with chrysophanol resulted in a 2-3 fold increase of DNA synthesis (Woelfle *et al.*, 1990).

Furthermore, chrysophanol exhibited antimutagenic activity against *Salmonella typhimurium* strain TA98, TA100; TA 1537and TA2637 (Choi *et al.*, 1997; Tikkanen *et al.*, 1983; Liberman *et al.*, 1980; Stark *et al.*, 1978) and antioxidant activity (Siddhuraju *et al.*,2002; Yen *et al.*,2000; Yuan and Gao, 1997).

Chrysophanol also showed antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* with MIC in the range 25 - 250 µg/ml (Palanichamy and Nagarajan, 1990b; Agarwal *et al.*, 2000). Chrysophanol also showed antiviral activity against poliovirus types 2 and 3 (Picornaviridae) *in vitro* by inhibition of the replication of virus (Semple *et al.*, 2001). Moreover, chrysophanol exhibited cAMP-dependent protein kinase inhibition activity, resulting in impairment of memory formation (Zhao *et al.*, 1995).

2.4.4 Emodin

Most reports on bioactivity of emodin have described its anticancer activity. This involves various mechanisms, including cytotoxic to mouse mammary carcinoma cell line, FM3A cells (Morita *et al.*, 1988); selectively blocked the growth of ras -transformed human bronchial epithelial cells (Chan *et al.*, 1993); block the G1 to S phase of the cell cycle of HCT-15 human colon carcinoma cells with (Kamei *et al.*, 1998); inhibit P-glycoprotein pump-efflux activity and reduced the expression of P-glycoprotein in MCF-7/Adr cells (Jiang *et al.*, 1999); induction of apoptotic responses in the human hepatocellular carcinoma cells (HCC) Mahlavu, PLC/PRF/5 and HepG2, lung carcinoma cells CH27 (human lung squamous carcinoma cell), human lung non-small cell carcinoma cells (H460), human promyelocytic leukemia cell line (HL-60) (Lee, 2001; Chen *et al.*, 2002; Jing *et al.*, 2002); inhibition of nitrite production from lipopolysaccharide (LPS)-activated mouse leukaemic monocyte macrophage cell (RAW 264.7) (Wang *et al.*, 2002); inhibit two-stage carcinogenesis test of mouse skin tumor (Koyama *et al.*, 2002) and inhibition of tyrosine phosphorylation of protein product of proto-oncogene (p185neu) overexpressed in cultured rat C611B cholangiocarcinoma (ChC) cells (Lai *et al.*, 2003).

Emodin also exhibited higher cytotoxicity against human oral squamous cell carcinoma (HSC-2) and salivary gland tumor (HSG) cell lines than normal human gingival fibroblasts (HGF) (Shi *et al.*, 2001). Emodin inhibited the growth of HER-2/neu-overexpressing tumors in mice and prolonged survival in mice bearing HER-2/neu-overexpressing human breast cancer cells (Zhang *et al.*, 1995; Chang *et al.*, 1996; Kim *et al.*, 1997; Zhang *et al.*, 1999). Moreover, it has been reported that emodin can sensitize these cells to chemotherapeutic drugs (Zhang and Hung, 1996). The combination of emodin and paclitaxel synergistically inhibited tumor growth and prolonged survival in athymic mice bearing subcutaneous xenografts of human tumor cells expressing high levels of p185neu (Zhang *et al.*, 1999). Furthermore, emodin markedly enhanced the cytotoxicity of 5-fluorouracil, mitomycin C (MMC) and methotrexate (MTX) against human hepatocellular carcinoma cell line (BEL-7402) and partly reversed the multidrug resistance in human breast cancer MCF-7/Adr cells (Jiang *et al.*, 1999). Emodin also showed suppressive effect on tumor invasion and metastasis (Zhu *et al.*, 2003). It has been reported that emodin at the concentration of 10 μg/ml inhibited human caucasian chronic myelogenous leukemia (K562) and Raji cell proliferations 97% and 98%, respectively (Kuo *et al.*, 1997).

Emodin also showed antifungal activity against *Trichophyton mentagrophytes* and *Microsporum canis* (Agarwal *et al.*, 1976); antimicrobial activity against *Staphylococcus aureus* and *Streptococcus viridans* with MIC of 6.25 - 12.5 and 25 - 50 μg/ml, respectively (Cai and Chen, 1988); antioxidant activity (Huang *et al.*, 1995; Yuan and Gao, 1997; Choi *et al.*, 2000; Yen *et al.*, 2000; Chiu *et al.*, 2002) antimutagenic activity (Lee and Tsai, 1991); antitricomonal activity against *Trichomonas vaginalis* in mice (Wang, 1993); laxative activity (Yang *et al.*, 1994) and hepatoprotective activity (Lin *et al.*, 1996; Zhan *et al.*, 2000).

However, emodin has been reported to show genotoxic and phototoxic activities, *in vitro* (Gross *et al.*, 1984; Grimminger and Witthohn, 1993; Lewis *et al.*, 1996; Mengs *et al.*, 1997; Mueller and Stopper, 1999; Mueller *et al.*, 1999; Vargas *et*

al., 2002). There are some reports on mutagenicity of emodin in the Salmonella/mammalian microsome assay (Ames test) with a specificity for strain TA97, TA100, TA102 and TA1537 (Bruggeman and van der Hoeven, 1984; Masuda and Ueno, 1984; Bosch *et al.*, 1987; Krivobok *et al.*, 1992). It has been shown that emodin possesses induction effect on cytochrome P450s 1A1 and 1B1 proteins and mRNA in human lung adenocarcinoma cells (CL5). Modulation of P450 by emodin may be an important factor affecting metabolism and toxicity of the hydroxyanthraquinone in humans (Wang *et al.*, 2001).

2.4.5 Rhein

Effects of rhein on electrolyte properties and Cl^- transport through prostaglandin-mediated effects on the apical Cl^- conductance of the colonic cells has been reported as a mechanism of its laxative activity (Clauss *et al.*, 1988). It has been demonstrated that rhein 6 mg% applied to the mucosal side of isolated rat colonic mucosa decreased the net absorption of Na^+, Cl^- and water in a dose-dependent manner (Wanitschke, 1980). Rhein activated chloride secretion by an excitation of submucosal neurons and release of acetylcholine and endogenous prostaglandins, but not by release of histamine or serotonin (Frieling *et al.*, 1993). In the stripped rat descending colon, rhein induced net secretion in the human jejunum and colon, and also decreased net absorption of electrolytes and water (Wanitschke and Karbach, 1988). In addition, rhein exerts its laxative effects by a mechanism that does not involve platelet activating factor (PAF) release (Tavares *et al.*, 1996).

Rhein has been reported to exert various biological activities against microorganisms. It exhibited antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* with MIC values of 25 - 250 μg/ml (Agarwal *et al.*, 2000). It also has antibacterial activity against *Bacillus megaterium, Escherichia coli, Bacillus subtilis, Micrococcus luteus, Clostridium perfringens* and *Fusobacterium varium*. It's minimum inhibitory concentrations against *Staphylococcus aureus* and *Streptococcus viridans* were

6.25 - 12.5 and 25 - 50 μg/ml, repectively (Matsumoto *et al.*, 1985; Cai and Chen, 1988; Tegos *et al.*, 2002). It has been reported that rhein exerts inhibition of arylamine N-acetyltransferase activity and growth of *Helicobacter pylori* isolated from peptic ulcer patients (Chung *et al.*, 1998). In addition, the use of rhein for treatment of oral infectious diseases has been reported (Didry *et al.*, 1994).

Rhein also possesses antioxidant activity, and exhibits inhibition of peroxidation of linoleic acid with a IC_{50} value of 64.2 μM (Malterud *et al.*, 1993; Yen *et al.*, 2000). Rhein also inhibits pyrogallol auto-oxidation and show free-radical scavenging activity against hydroxyl radicals (Yuan and Gao, 1997).

Anticancer activity of rhein with different mechanisms has also been reported. The action mechanisms include interference with the stimulating effect of K^+ of leukocytes membrane (Mian *et al.*, 1987); inhibition of neoplastic growth through ATP depletion (Floridi *et al.*, 1992); inhibition of glucose transport in Ehrlich ascites tumor cells by alteration of membrane-associated functions (Castiglione *et al.*, 1993); interference with tumor cell proliferation by affecting energy metabolite and mitochondrial function (Iosi *et al.*, 1993) and inhibition of 12-O-tetradecanoylphorbol-13-acetate (TPA) induced cell transformation and activator protein-1 (AP-1) activation in a dose-dependent manner in mouse epidermal cell line (JB6) (Lin *et al.*, 2003). *In vivo* study showed that rhein, at the dose of 40 mg/kg/day given by intraperitoneal injection for 7 days, markedly increased the survival time of the mice with P388 leukemia. The ascites volume and tumor cell number were also decreased by inhibited of the biosynthesis of DNA, RNA, and protein of P388 leukemia cells (Lu and Chen, 1989). However, the *in vitro* treatment of primary rat hepatocytes with rhein resulted in a 2-3 fold increase of DNA synthesis; these results suggest that rhein may also have tumor-promoting activities (Wolfle *et al.*, 1990).

Antiviral activity of rhein against a normal laboratory human cytomegalovirus (HCMV) strain, AD-169 has also been reported as well as cytotoxicity (Barnard *et al.*,

1992) and decrease the survival fraction of cultured human glioma cells (Floridi *et al.*, 1990).

2.5 Anthraquinone production by plant cell and tissue cultures

The development of plant tissue cultures as an alternative source of secondary metabolites from medicinal plants is an interesting research field. In addition, some plant tissue cultures have been reported to produce new compounds, for example the formation of new indole alkaloids formation in *Rauvolfia serpentina* hairy root cultures (Sheludko *et al.*, 2002a; Sheludko *et al.*, 2002b). Anthraquinone is a group of secondary metabolites that have been reported to be produced by several plant tissue cultures as shown in Table 2.2.

Many techniques have been successfully used for improving secondary metabolite production by plant tissue cultures (Dixon, 1991; Sahm, 1993; Narayanaswamy, 1994), including:-

- Medium manipulation

The classical approach to improve secondary product accumulation in plant tissue cultures is to manipulate the medium with a range of plant growth regulators, mineral salts and carbon sources, or environmental conditions.

- Selection of high yielding plants

Each plant can show different rates of secondary metabolite formation. The explant from high yielding plant will be high producing cultures.

- Elicitation

Elicitation is the induction of secondary metabolites by the addition of a natural or denatured preparation of a fungus or microorganism, either complex or some time reduced to a defined polysaccharide preparation. The secondary

compound induced is called a phytoalexin and is regarded to be a defense compound directed against an invading pathogen.

- Immobilization

The immobilization process has a positive effect on the production of secondary metabolites, partially due to higher synthetic rates, partially due to longer production periods. This method is entrapment of the cell culture within a gel (calcium alginate, agar, polyacrylamide), a solid support or both.

Table 2.2 Examples of anthraquinone production by plant tissue cultures

Plant	Anthraquinone	Tissue culture Technique	Reference
Cassia acutifolia	anthraquinones	medium manipulation	Rady and Nazif, 1997
Cassia acutifolia	sennoside	medium manipulation	Nazif *et al.*, 2000
Cassia obtusifolia	anthraquinones	elicitation	Guo *et al.*, 1998
Galium vernum	anthraquinones	immobilization	Strobel *et al.*, 1991
Galium vernum	anthraquinones	immobilization and three phase system	Dornenburg and Knorr, 1996
Morinda citrifolia	anthraquinones	elicitation	Dornenburg and Knorr, 1994
Morinda citrifolia	anthraquinones	medium manipulation	Hagendoorn *et al.*, 1994
Morinda citrifolia	anthraquinones	two phase system	Bassetti *et al.*, 1996
Morinda citrifolia	anthraquinones	medium manipulation	Hagendoorn *et al.*, 1997
Morinda elliptica	anthraquinones	medium manipulation	Abdullah *et al.*, 1998
Morinda elliptica	anthraquinones	bioreactor	Abdullah *et al.*, 2000
Rheum palmatum	anthraquinones	medium manipulation and elicitation	Chang *et al.*, 1998
Rheum ribes	anthraquinones	medium manipulation	Sepehr and Ghorbanli, 2002
Rubia akane	1,2-dihydroxy-anthraquinone	medium manipulation	Mizutani *et al.*, 1997

Table 2.2 (cont.)

Plant	Anthraquinone	Tissue culture Technique	Reference
Rubia akane	anthraquinones	elicitation	Shim *et al.*, 1999
Rubia cordifolia	munjistin, purpurin	vary explant	Mischenko *et al.*, 1999
Rubia cordifolia	alizarin, purpurin	elicitation	Shin and Lyu, 2000
Rubia cordifolia	anthraquinones	elicitation	Bulgakov *et al.*, 2002
Rubia tinctorum	nordamnacanthal	elicitation	Heijden *et al.*, 1994
Rubia tinctorum	anthraquinones	elicitation	Mantrova *et al.*, 1999